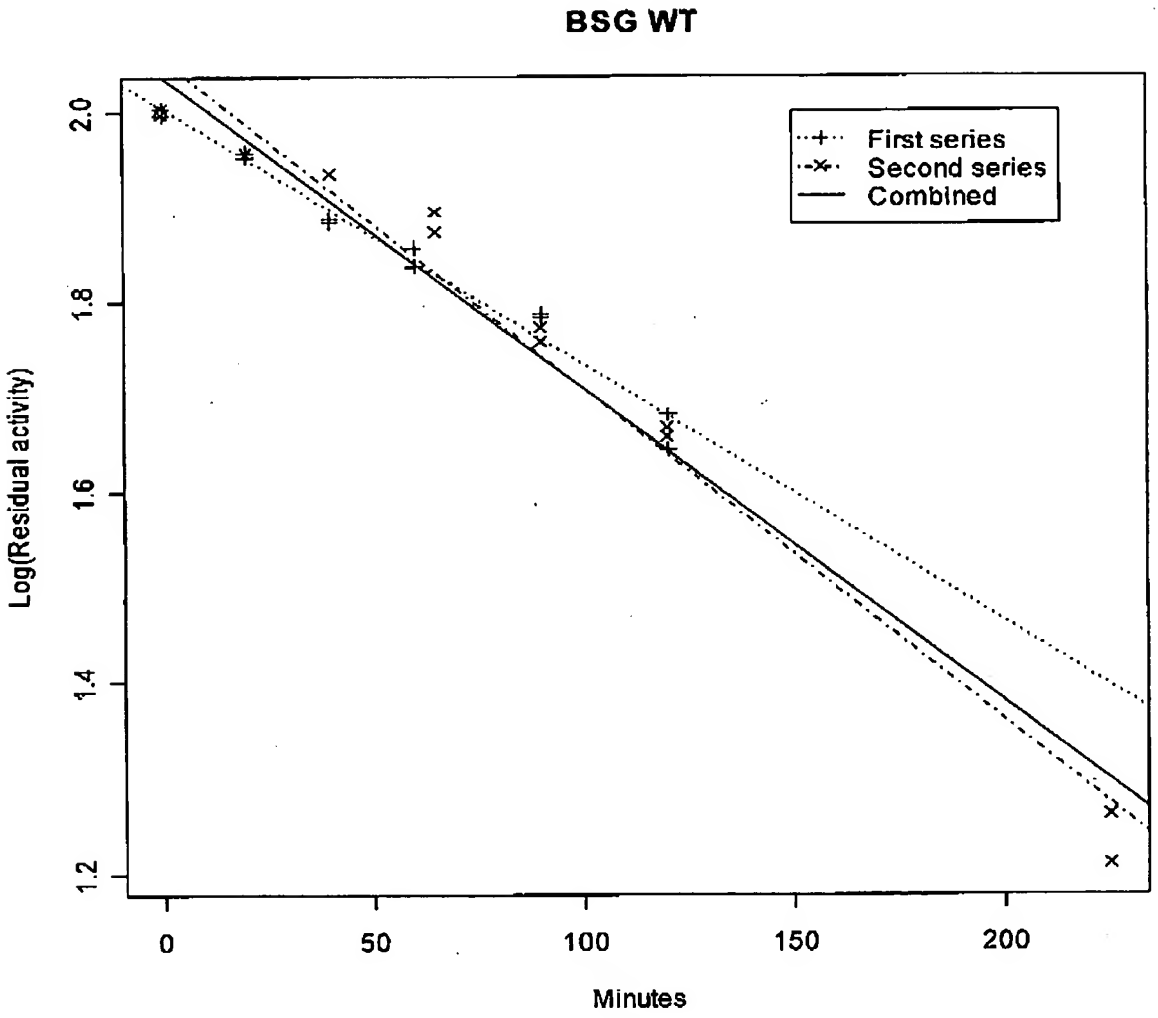
Appendix 1:

Differences between data-series, BSG:

BSG-WT

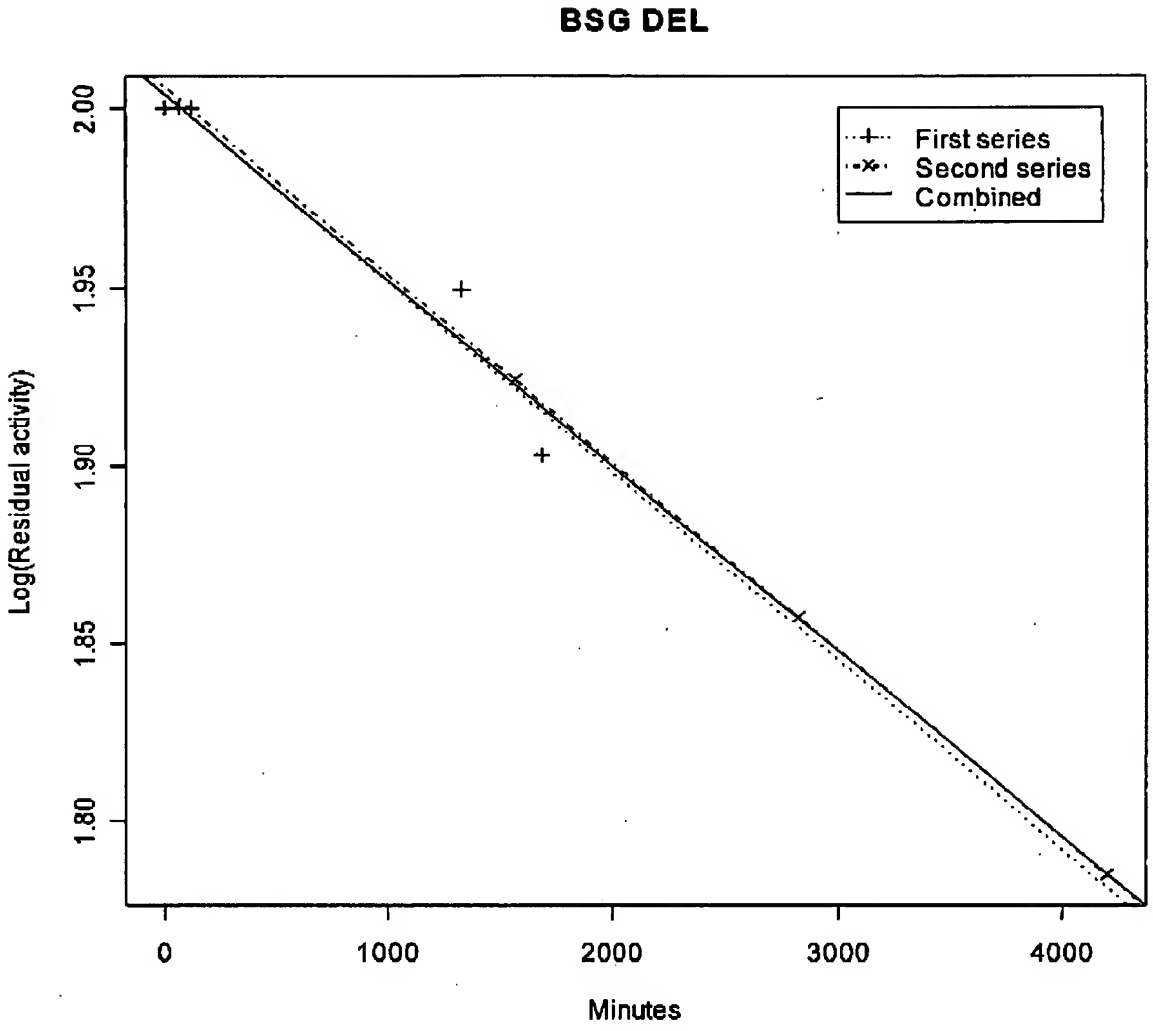


Summary of statistical analysis.

Below is a screen-dump from the statistical analysis, showing that there is a significant difference in the slope in the two data series. The p-value for same slope is underlined.
The analysis was done in R version 1.8.1 (http://www.r-project.org). The data for the BSG WT is held in the data-frame `bsg.wt` as shown in the table above. In the data-frame the time is called `var1`, the residual activity is `var2` and `var3` is a factor over the two series of experiments. The output shows the effect of the factor on a linear regression on the Log(residual activity) over incubation time.

```
> summary(lm(log10(bsg.wt$var2)~bsg.wt$var1*bsg.wt$var3))

Call:
lm(formula = log10(bsg.wt$var2) ~ bsg.wt$var1 * bsg.wt$var3)

Residuals:
     Min       1Q   Median       3Q      Max
-0.063233 -0.012558  0.001456  0.020149  0.063980

Coefficients:
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)              2.0043563  0.0165332 121.233  < 2e-16 ***
bsg.wt$var1             -0.0027017  0.0002416 -11.183 4.68e-10 ***
bsg.wt$var32             0.0520051  0.0226679   2.294   0.0327 *
bsg.wt$var1:bsg.wt$var32 -0.0007776  0.0002771  -2.806   0.0109 *
---
Signif. codes:  0 `***' 0.001 `**' 0.01 `*' 0.05 `.' 0.1 ` ' 1

Residual standard error: 0.03408 on 20 degrees of freedom
Multiple R-Squared: 0.9768,     Adjusted R-squared: 0.9733
F-statistic: 280.4 on 3 and 20 DF,  p-value: < 2.2e-16
```

BSG-DEL



Summary of statistical analysis.

Below is a screen-dump from the statistical analysis, showing that there is not a significant difference in the slope in the two data series. The p-value for same slope is underlined.
The analysis was done in R version 1.8.1 (http://www.r-project.org). The data for the BSG deletion is held in the data-frame `bsg.del` as shown in the table above. In the data-frame the time is called `var1`, the residual activity

is var2 and var3 is a factor over the two series of experiments. The output shows the effect of the factor on a linear regression on the Log(residual activity) over incubation time.

```
> summary(lm(log10(bsg.del$var2)~bsg.del$var1*bsg.del$var3))

Call:
lm(formula = log10(bsg.del$var2) ~ bsg.del$var1 * bsg.del$var3)

Residuals:
       1        2        3        4        5        6        7        8
-0.0043196 -0.0008682  0.0020522  0.0151601  0.0002194 -0.0120245 -0.0004197
0.0002003

Coefficients:
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)               2.004e+00  5.978e-03 335.303 4.75e-10 ***
bsg.del$var1             -5.310e-05  6.243e-06  -8.505  0.00105 **
bsg.del$var34             1.836e-03  1.739e-02   0.106  0.92103
bsg.del$var1:bsg.del$var34  4.731e-07  8.218e-06   0.058  0.95685
---
Signif. codes:  0 `***' 0.001 `**' 0.01 `*' 0.05 `.' 0.1 ` ' 1

Residual standard error: 0.009979 on 4 degrees of freedom
Multiple R-Squared: 0.9905,     Adjusted R-squared: 0.9834
F-statistic: 139.2 on 3 and 4 DF,  p-value: 0.0001682
```

Comparing thermo stabilization.

Analysis of significance of different stabilization:

We have the following slopes on the curves:

	Slope	Std err	Relative std err
BAN WT	-0.346932	0.042031	0.121152
BAN DEL	-0.031550	0.001153	0.036556
BSG WT	-0.003286	0.000128	0.039024
BSG DEL	-0.000052	0.000002	0.040217

We can compute the ratios of the slopes (which are the reciprocals of the ratios of the half-lifes).

	Ratio	Std err	Relative Std err
BAN WT / BAN DEL	10.9962346	1.3915416	0.1265471
BSG WT / BSG DEL	63.04430515	3.53290004	0.05603837

This means we have a ratio between the slopes of

$$\frac{63.0}{11.0} = 5.73$$

with a relative standard error of

$$\sqrt{0.127^2 + 0.056^2} = 0.138$$

and a standard error of

$$5.73 * 0.138 = 0.79$$

So, if we use the golden rule of standard errors, that the true value is within +/- two standard errors of the estimated value, we have that **the deletion has a stabilizing effect in BSG which is between 4 and 7 times what is seen in BAN.**

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Fall 2001	DIEU: Assertionstræning
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US 6,159,688 (method of producing polynucleotide variants)
US 6,165,718 (method for in vivo production of a mutant library in cells)
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